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# On the Structure and Development of the Tubular Enamel of the Sparidae and Labridae

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# VII. *On the Structure and Development of the Tubular Enamel of the Sparidæ and Labridæ.*

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[Plates 5 and 6.]

The present investigation has been undertaken in order to endeavour to determine the tubular nature of the striæ from without, in the enamel of certain fish, and their functional significance, and to show that, in the Sparidæ and Labridæ, the mode of development of the enamel differs in essential particulars from that hitherto described in fish, a regular penetration by vascular tubes and a secreting structure in the enamel organ being demonstrable.

A penetration of the enamel by tubes from the dentine is very commonly found in fish, and a strongly marked striation from the outer surface, which does not reach the dentine, is also seen in many groups.

In the Plagiostomi, it is seen in *Cestracion* (*Heterodontus*), *Lamna*, and in many sharks both recent and fossil, and among osseous fish in the Gadidæ, Labridæ, and Sparidæ or sea breams, in the latter being especially well marked in the group Sargina, the enamel of a member of this group, the *Sargus ovis*, or sheep's head fish of the Atlantic coasts of North America, having long been taken as the typical example of this penetration from without. Such markings or striæ from the outside of the enamel have hitherto never been conclusively shown to be of a tubular nature, and Mr. Charles Tomes says (1A) that in all these examples in fish in which "there is a distinct striation which starts inwards from the free surface and is lost before it reaches the dentine surface, it remains somewhat doubtful whether these striæ are tubes or merely very distinctly marked-out solid prisms, which serve to make this peculiar and characteristic pattern." He also says he has never been successful in getting coloured fluids to enter the striæ of these enamels. In the present paper I shall endeavour to show that these markings are produced by a regular system of tubes, and that they are quite independent of the prisms of the enamel.

With regard to the nature of the outer layer of the teeth in the Plagiostomi, there has been considerable difference of opinion, Rose (2) looking upon it as dentine, but Mr. C. Tomes considering, on the whole of the evidence afforded by the structure and development of this tissue in Elasmobranchs, that it may appropriately

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be called enamel (1B). He showed that, "while the organic matrix of the enamel in these fish is beyond question furnished by the mesoblastic dentine papilla, the epiblastic ameloblasts over it are in a state of development, which implies that they take an active part, and that the tissue is a joint production," and, in his classification of enamels, he places it under the heading of enamels which are not wholly epiblastic, "a tissue which is laid down by the operation of epiblastic ameloblasts in a matrix which is derived from the mesoblastic dentine papilla."

In Elasmobranchs there is often a very distinct striation from without in this enamel-like layer, as well as a penetration from the dentine side, whether this be osteo-dentine or tubular dentine.

In *Heterodontus*, where the actual line of demarcation between the dentine and the enamel-like layer is very imperfectly defined, the surface of this outer layer is marked by little pits or depressions, and a longitudinal section shows radiating lines from the bottom of these pits passing into it.

I have succeeded in procuring a thorough staining of these pits and striæ with alcoholic stains, by which they are very strongly coloured, and a broad band of more diffuse stain passes a little way into the enamel at the base of the depressions. The large tubes of the osteo-dentine, which forms the bulk of the tooth, give out tree-like branches, which take the stain freely, and divide and subdivide, their ultimate fine ramifications passing outwards and crossing and mingling with the stained fine tubular prolongations from the outer surface. Fine tubes also pass in from all parts of this surface, although larger and more deeply penetrating in the radiating bundles which start from the bottom of the depressions or pits. The two systems of tubes in these preparations certainly appear to communicate with one another on the inner margin of the enamel-like layer by their finest branches.

The teeth of *Lamna cornubica* and of several other sharks, treated with these stains, show a distinct penetration by stained tubes from the outer surface.

The penetration of the stain in this enamel-like layer of the Plagiostomes would serve to indicate that these markings, hitherto described as striæ, form a true tubular system, and would tend to suggest a still closer analogy with the true enamel of the osseous fish, and to confirm Mr. C. Tomes' view with regard to its nature.

### *The Enamel of* Sargus.

In the Sparidæ the teeth are confined to the intermaxillary and premandibular bones. The incisors in *Sargus* bear a remarkable outward resemblance to human incisor teeth, but are firmly anchylosed to the bone of the jaw. The rounded molars are arranged in several rows, the number of which varies in the different species, and in many the outer row of the molars anteriorly are somewhat conical, the teeth in the front of the mouth being adapted for cropping the marine algæ, and the rounded molars for the crushing of hard-shelled animals, which form part of the food of these fish.

In ground sections of the incisors or molars of *Sargus ovis*, the tubes are seen to pass into the enamel at right angles to its surface, and about half-way across its width bend right and left at an obtuse angle, crossing one another on the inner third, and terminating at a line of dense calcification, which forms a dark band, following the contour of the dentine surface, but separated from it by a narrow clearer space. The tubes do not reach the dentine in any part. The very complicated pattern shown and described in *Sargus* enamel is only due in part to the ramifications of the tubes, which can be easily followed in the stained preparations and distinguished from the prisms, for the densely calcified band of enamel near the dentine is formed by the exceedingly intricate and complicated course of these prisms, and contains no tubes from without.

The prisms of the enamel are left unstained, and, being thus distinguished from the tubes, show that the striæ and the enamel columns are not identical, as has been suggested.

This is clearly shown in the stained preparations, as the red-stained tubes are relieved strongly against the unstained yellowish enamel, which in the area bordering the dentine seems to be made up of prisms passing in every direction and spirally twisted, and it is quite impossible to follow the course of individual prisms. This pattern, shown in Plate 5 (figs. 1, 2, 3, 4, at *e'*), is formed in the abundant organic matrix which is laid down in early stages of the development of the enamel, before any calcification has commenced either in the enamel or dentine, as will be shown later.

At the outer margin of the enamel between the stained tubes, a longitudinal striation is visible at right angles to the surface, formed by the columns of the unstained enamel prisms, which in this situation are regularly arranged (Plate 5, figs. 2, 3). A marked cross striation or banding of the enamel columns can also be seen; these bands vary in width and conform to the contours of the outer surface of the enamel, and suggest that they are due to incremental deposit, the outermost bands being broader than those deeper in, and taking the red stain very faintly.

In preparations cut from specimens which had been preserved in formalin while in the fresh state, the contents of the tubes are very apparent as minute granules (Plate 5, fig. 4, *a*). In several of the wide stained tubes and uncalcified spaces in these preparations small deeply stained round bodies are seen as well as the dark granules. These small stained bodies bear a very strong resemblance to the nuclei of the blood corpuscles. It is difficult to account for their presence in the enamel, unless we suppose that in this last stage of calcification, just before the eruption of the tooth, some blood corpuscles find their way into these spaces from the vascular tubes of the enamel organ to be presently described.

The tubes from the outside vary considerably in size, and some are so tightly packed with granules that the stain is unable to penetrate completely. The interlaced terminal branches of the enamel tubes terminate somewhat abruptly at the




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upper margin of the dense band of enamel prisms before described (Plate 5, fig. 4).

In another species, *Sargus noct* (Ehrenberg), a Mediterranean and Red Sea fish, there is a still more complicated pattern to be seen in the enamel than in that of *Sargus ovis*. In this species there is a very free and regular penetration of the enamel by tubes from the dentine as well as by the large tubes from the outer surface. These dentinal tubes enter in separate radiating bundles, the tubes in each little bundle first curving outwards, then inwards towards one another, finally spreading out all across the enamel, many terminating close to its free surface (Plate 5, figs. 1, 2, 3). Some of these tubes from the dentine can be distinctly traced into elongated, stained dilatations, which appear to be the remnants of the outer system of tubes closed in by calcification (Plate 5, fig. 3).

These two separate systems of tubes, crossing one another in many directions, form, with the twisted and spirally arranged enamel prisms, a very intricate and complicated pattern. Many of the dentinal tubes divide and branch, as do also those from the outside, but the majority of the tubes of both systems pass in more or less open curves, interlacing with one another. The general trend of both sets of tubes, one from the dentine, the other from the surface, is easily distinguished.

In the fully erupted and functional tooth of this species, many tubes belonging to the outer system appear to be shut off from the outer side of the enamel, and the dentinal tubes predominate, although here and there some large tubes, or bundles of tubes, from the outside are seen entering.

The festooned outline of the enamel towards the dentine appears to indicate, as at the enamel junction in human teeth, that the calcifying substance is deposited in globular forms producing these contours between the bundles of tubes.

In *Sargus ovis*, the tube system from without is very fully developed, and in the fully formed tooth, nearly all the tubes are seen to communicate with the exterior, and take the stain freely (Plate 5, fig. 4). Von Boas, in his paper on the teeth of the Scaroids (3), says that the enamel tubes in *Sargus* which show a similar condition to that in Scarus do not open on the surface ("Die Röhren münden nicht an der äusseren Oberfläche des Schmelzes").

Although in unstained preparations it may be somewhat difficult to be quite certain on this point, an examination of stained specimens makes it very evident that they do open on the surface, and a study of unerupted teeth treated in the same manner, as I shall presently show, conclusively proves that this emphatic statement of von Boas was due to an error of observation.

This penetration by both sets of tubes does not appear to have been hitherto described in *Sargus*, as the species examined had been *Sargus ovis*, in which there is no penetration by the tubes of the dentine. It is interesting to note an intermediate form of tubular enamel in the genus *Sargus*, which appears to form a link between the species above described and *Sargus ovis*. In an incisor tooth of *Sargus vulgaris* there

is penetration by both systems of tubes, but although the dentinal tubes are collected into little bundles where they enter the enamel, they pass in but a very short distance, terminating in the line of dense calcification where the prisms form the complicated pattern before described (Plate 5, fig. 5). The bundles of tubes from the dentine do not, however, show the same graceful curves seen in *Sargus noct*, but spread suddenly out in a radiating manner. In another (dry) specimen of *Sargus* which I also examined, and which the characteristics of the teeth indicate to be probably also allied to *Sargus vulgaris*, the incisor teeth do not show any entrance of the dentinal tubes into the enamel, but the molar teeth show it very distinctly, and the arrangement of the tubes is identical with that seen in the specimen of *Sargus vulgaris*.

We see, therefore, in the same genus (*Sargina*) :—

(1) A very complete penetration by both systems of tubes in *Sargus noct*.

(2) A partial penetration from the dentine and a full one from the outside in *Sargus vulgaris*.

(3) A complete suppression of the dentinal tubes in *Sargus ovis*, only the tubes from without being present.

This appears to be contrary to the generally received opinion that differences in the structure of the enamel do not occur within the same genus.

A specimen of *Chrysophrys* showed an exactly similar arrangement of the tubes of the enamel to that seen in the molar teeth of *Sargus ovis*. The stain penetrated the tubes freely from without, but the dentinal tubes did not enter the enamel. A section of the enamel of this *Chrysophrys* could not be distinguished under the microscope from one of *Sargus ovis*.

A member of another group of the Sparidæ, *Pagellus lithognathus*, shows a very similar structure to that of *Sargus noct*. The stain penetrates the tubes of the enamel from without, and also others from the dentine which pass in in bundles and penetrate the enamel deeply. They radiate out from the point of entry as in *Sargus vulgaris*, and have not the curved arrangement of those of *Sargus noct*.

In *Sargus* the teeth replace one another vertically, the successional teeth lying immediately beneath those in use and within the substance of the bone.

When the functional tooth is removed (in formalin-preserved preparations), the crown of the tooth beneath is exposed, but is seen on close examination to be covered with a thin pellicle of the enamel organ.

If the enamel of these unerupted teeth is stained with alcoholic fuchsin and examined, it is seen to be very different in appearance from the erupted teeth. The tubes from the outside have very open orifices, and the enamel is stained deeply in very broad vertical stripes (Plate 5, fig. 1). These broad bands pass about halfway across, and then become fused in a deeply stained area which extends horizontally across the enamel, being sharply limited at the lower margin by the dense calcified layer near the dentine, before described.

If a tooth be examined which is a little further advanced towards eruption (Plate 5, fig. 2), the stained horizontal band is seen to be broken up, only a few patches of stained material being seen in its former position, but many of the entering tubes are still very wide and show laterally expanded areas.

All stages of this gradual contraction of the stained areas in the enamel are to be seen in the molar teeth in different stages of development to the completed enamel of the tooth in wear.

Both incisors and molars are found in *Sargus*, in which the enamel has a translucent appearance, and these cut in the microtome with the same ease as ordinary uncalcified tissue. All these little caps of translucent enamel appear to be of about the same thickness, which is very much less than that of the completed enamel. This has a strong bearing on the mode of its development, to which I shall presently refer. Sections of these enamel caps stain throughout, and exhibit the same complicated pattern described in the prisms of the completed tooth near the dentine.

From these appearances, I think, it is impossible to avoid the conclusion that the tubes have a calcifying function, conveying the lime salts to the organic foundation substance of the enamel. The stained spaces in the unerupted tooth are enormously wider than the largest tubes of the enamel; they must be, therefore, spaces of incomplete calcification into which these tubes lead. The explanation of these appearances is, I think, to be found in the different stages of development seen in the enamel organ of these fish, and to the fact that the pellicle of the enamel organ, which, as I shall presently show, has in its later stages a free supply of blood-vessels, remains attached to the surface of the enamel until it is actually fully erupted and in wear.

### *Development of Enamel in* Sargus.

Mr. C. Tomes (1A) showed that, in the Gadidæ, and, as he says, probably in other osseous fish, there is a very marked difference in the mode of development of the enamel from that found in mammalian enamel organs.

The enamel organ is at first similar in appearance to that of the Mammalia, although showing no stellate reticulum or stratum intermedium, but the amelobasts soon disappear, and the enamel organ is converted into a stroma traversed by tube-like prolongations, the part near the forming enamel being changed into a more delicate stroma, in which the tubes of the enamel organ are not seen.

He was unable to obtain satisfactory preparations of *Sargus* and *Labrus*, owing to the great difficulty in procuring properly hardened material, the germs being so deeply seated in the hard bone of the jaw, but those he did procure he considered indicated that the process was the same as in the Gadidæ. There are, however, modifications in the mode of development in *Sargus*, and especially in *Labrus*, of a very strongly marked and distinctive character.

Mr. Tomes considered that, for the reasons he gave in his paper (1A), the difficulties in obtaining satisfactory preparations of *Sargus* and *Labrus* were "almost insuperable," but it seemed that, if the germs could be exposed in the fresh fish and placed at once in the fixing solution, the difficulties might be overcome.

Through the kindness of Dr. Cryer, of Philadelphia, I was able to obtain specimens which he had preserved for me in this manner, and they gave excellent results. This was not, however, necessary in the specimens of *Labrus* examined, where the penetration by the fixing solution was quite satisfactory without this precaution, the germs not being situated in such dense bone as in *Sargus*.

These specimens, when decalcified, gave some good preparations, although not so perfect as later ones from the same material, where the use of paraffin and alcohol was avoided, and a few fragments of calcified enamel which had escaped the full action of the acid remained attached to the stroma. These portions of enamel are seen at *e* in fig. 9, Plate 5—the part between these and the dentine having been entirely removed by the decalcifying agent.

It is seen that processes extend from the capsule (Plate 5, fig. 9), where they are apparently in contact with the connective tissue layer, to the outer surface of the stroma (*s*), enclosing a reticular tissue between them. No cells of the external epithelium are now visible.

Examination with a high power shows that fine striæ, lying more or less parallel to one another, run into and become incorporated in this forming enamel (Plate 5, fig. 12), but the large tubes of the enamel organ do not enter it. This portion of the enamel, however, shows orifices and apparent perforations, as if it were penetrated by tubes, but these are apparently due to spaces left in the weaving, as it were, of the threads of the stroma in the forming enamel, and not to the penetration of large visible tubes.

Some earlier tooth germs, however, exhibited a different condition. In these the dentine pulp is very large, and a very distinct layer of fully differentiated odontoblasts is seen at its periphery, but no dentine at all is formed, and the tissue lying in contact with the layer of odontoblasts is enamel, deeply stained, and showing with great distinctness the remarkable twisting and intermingling of its fibres, described above, in the part of the calcified enamel near the surface of the dentine, and in the little translucent enamel caps, which become detached from the enamel organ in many preparations. In this instance it has fortunately remained in position, and in contact with its outer surface is a distinct layer of nucleated ameloblasts (Plate 5, fig. 8), and there is no appearance of tubes in the enamel organ or in the forming enamel.

These paraffin sections were, unfortunately, disturbed in cutting, and, all being cut from the same block of paraffin, showed the same disturbance. A layer of ameloblasts, however, remains attached at one place to the forming enamel, which also shows, where not hidden by the cells, thread-like projections, which have

become incorporated in it, and which are apparently portions of the stroma in which the ameloblasts lie, and probably a product of these cells—a delicate reticulation is also seen between the ameloblasts and the connective tissue boundary of the enamel organ (Plate 5, fig. 8, S). It would appear as if this were the first indication of the formation of the stroma, into which the whole of the ameloblasts become afterwards converted.

In the undisturbed position of the tooth germ, this stained layer of enamel must have occupied its whole area, as the continuation of the layer of ameloblasts is seen at the periphery beneath the external layer of the enamel organ. It would thus appear that this first-formed portion of the enamel is produced in the presence of true enamel cells, and it is seen that the ameloblasts in *Sargus* do not disappear until this first layer of enamel is formed. After this stage has been reached, the ameloblasts, as well as the cells of the external epithelium, are no longer seen, and the tubes and the delicate stroma have taken their place. While the ameloblasts are present, there is no tubular structure visible in any of the germs examined, and it is especially noticeable that this first-formed portion of the enamel laid down by the ameloblasts is not tubular in *Sargus ovis*, where it is not crossed, as in *Sargus noct*, by dentinal tubes.

Some preparations cut in the freezing microtome showed a very interesting condition in the tubular portion of the enamel organ in the later stages of development. In the paraffin sections, the tubes at the circumference did not show definite structure with any distinctness, nor did they stain well, the heat employed in paraffin embedding appearing to be very injurious to the delicate enamel organs of fish. The frozen sections, however, showed no appreciable disturbance of this portion of the tooth germ, and they stained well with both methyl eosin and Weigert's iron hæmatoxylin.

It is seen in Plate 5, fig. 10, that vascular tubes or spaces are continuous with the blood-vessels of the capsule, that they pass inwards to the stroma and terminate in blunt endings or in apparent loops in this situation. The tissue between the vascular tubes, which was only vaguely indicated in the paraffin sections, is seen to consist of tube-like processes which do not extend to the capsule, but their free ends are seen between the vascular tubes slightly separated from the blood-vessels of the capsule, which are present in great abundance (Plate 5, fig. 10). These processes, when followed down, are seen to broaden out and become fused with the reticulate stroma in which the enamel is calcified. Numerous ovoid and irregularly shaped bodies are seen in the stroma which take a uniform stain and have no resemblance to cells, and are in all probability the calcoglobulin basis of the lime spherites laid down in the stroma. Large isolated polygonal nucleated cells are also seen scattered in the stroma.

The vascular tubes may perhaps be better described as sinuses containing blood rather than as true tubes. There are no endothelial cells visible in the delicate

confining tissue which forms their walls, and they are marked only by a fine longitudinal striation. The appearance of isolated, deeply stained masses appears to be due to an aggregation of the blood corpuscles caused by clotting. In *Halichœres*, as presently described, they appear as very greatly dilated spaces filled with blood corpuscles with a scarcely demonstrable limiting membrane (Plate 6, fig. 22). In many slides of *Halichœres*, these blood-containing spaces are many times wider than the tubular processes between them.

The vascular tubes, where they enter the enamel organ, are continuous with the blood-vessels of the capsule, but do not enter the stroma, terminating between the processes which I have described as being prolonged into the stroma.

On the other hand, the processes which enter into and become blended with the stroma do not reach the outer margin of the enamel organ, but their free extremities are seen between the vascular tubes in this situation.

These processes have an irregular outline, with concavities on all sides (Plate 5, fig. 11, *t*), and the margins of the concavities form projections, which communicate with the blood-containing tubes forming ovoid spaces or alveoli containing very delicate granules and small, round, deeply staining nuclei. The processes appear to be made up of ovoid bodies arranged more or less longitudinally, which, under high magnification, show no nuclei or definite structure. The appearances suggest that the centres of these processes serve as ducts, which, I think, after an examination of the process of development in *Labrus*, is the true explanation of their function.

We thus see that in the later stages of development in *Sargus*, blood-vessels are present in the enamel organ, which enter it at right angles to the surface, have a regular arrangement, and enclose a cellular and tubular tissue between them, and if these cells and blood-vessels have a secreting function, as seems highly probable, we have an apparently satisfactory explanation of the appearances in the teeth which have not fully erupted (Plate 5, figs. 1 and 2). The transverse stained band in the unerupted tooth is seen to be in contact at its lower margin with the inner layer of enamel, the whole or certainly the greater part of which has been laid down by ameloblasts before any dentine is produced, and it extends no further than this layer. This stained band is evidently an area of very imperfect calcification, its organic matrix has been laid down in the stroma of the enamel organ, and the calcifying salts would appear to be conveyed to it through these wide open tubular spaces in the enamel with which it communicates.

Although, as I have stated, the enamel in these unerupted teeth is apparently exposed when the molar above it is removed, it is covered with a thin pellicle of the enamel organ, which, on being detached and examined under the microscope, is seen to be made up of tubes and blood-vessels in parallel rows, with small and large cells, some of them filled with granules, lying between them. So long as this pellicle is in contact with the surface, the enamel would be able to receive a further




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impregnation with lime salts through the agency of the tubes and cells which are in connection with the circulating blood.

*Development of the Enamel in Labridæ.*

The species examined were :—*Tautoga onitis*, the black fish of the North Atlantic American coasts, preserved in formalin ; *Halichœres poœcilopterus* and *Pseudolabrus japonicus*, from Japan,* preserved in spirit.

*Tautoga onitis.*—The most instructive sections were obtained from *Tautoga*. Both the pharyngeal teeth and those from the intermaxillary and premandibular bones were examined, and gave similar results. The pharyngeal bones are easily decalcified, and are crowded with germs in all stages of development.

The appearance of a tubular secreting structure in the enamel organ seems to mark the later stages of calcification in *Sargus*, but in *Labrus* we find at very early stages indications of such a structure.

In sections of the earliest germs of *Tautoga*, the crescentic enamel organ over the dentine papilla consists of two layers of nucleated cells, which are continuous with one another at the horns of the crescent ; these apparently correspond to the internal and external epithelium in Mammalia, there being no stratum intermedium or stellate reticulum. In a more advanced germ, where the two layers of cells are slightly separated, at the upper and central part of the crescent, a small mass of glandular tissue is seen in contact with the outer layer of cells (Plate 6, fig. 13, *g*), and a slight deposit of uncalcified tissue is seen between the ameloblasts and the odontoblasts.

At a still later stage (Plate 6, fig. 15), the glandular tissue is seen to have become blended with the cells of the external epithelium, and is spreading around the outer circumference of the enamel organ, and enclosing it very much as the first epithelial inflection encloses and surrounds the dentine papilla. At this stage only a few blood-vessels are seen within the capsule.

Still later germs show a very regular arrangement of the blood-vessels, which run in tubular prolongations (Plate 6, fig. 14, *e.o.*). Although these vascular tubes are arranged in even parallel lines at right angles to the surface, the blood-vessels are seen in many places to pass transversely to the surface, and sections of them are seen within the tubes, which would seem to indicate either that they take a twisted course within the tubes, or that the apparent tube is a continuous sheath to the blood-vessels which is cut across. This sheath is very clearly seen in fig. 18, where two vascular tubes are separated from the enamel organ. The ameloblasts and external epithelium have now entirely disappeared, and connective tissue and blood-vessels only intervene between the enamel organ and the bone of the capsule.

* The specimens from Japan were kindly given me by Mr. C. T. Regan, F.R.S., of the British Museum of Natural History, to whom I am also indebted for assistance in the identification of species.

Between the parallel vascular tubes what appear to be distinct tubular glands are seen, which form a perfectly even and regular row around the enamel organ when cut in the correct plane of the section (Plate 6, figs. 16 and 17). Where these glandular structures are in contact with the capsule, they are seen to be enclosed by a distinct basement membrane, and the elongated nucleated cells open into a central duct or ducts, which pass in a direct line to the margin of this layer, where it is in contact with the stroma of the enamel organ. The glandular layer ceases abruptly in this position in a well marked curved line, for the delicate stroma is nearly always detached and carried away with the portions of decalcified enamel. In a few places, however, portions of the stroma are still in contact with this layer, and in several sections of the germ of *Halichœres*—which were not fully decalcified—the secreting tubes, the stroma, and the calcified enamel are seen in undisturbed connection (Plate 6, fig. 22).

Examination of this layer in *Tautoga* with a high power shows three or four ducts in the centre of the tubular structures, which contain rows of granules. In fig. 17 they are seen to run parallel with those from the neighbouring gland, and to open on the under surface of the enamel organ in slightly radiating bundles, the intervals between the bundles of ducts being filled up by a tissue consisting of delicate fibres lying parallel with them. The glandular tissue closely invests the blood-vessels, and where cut across can be seen surrounding them. In some places the ducts have a distinct corkscrew form.

As seen in figs. 13 and 15, this glandular formation is not produced by any involution of the cells of the enamel organ, either of the ameloblast layer or that of the external epithelium, but is a separate tissue which invades the capsule from without, and, as shown in many preparations, is derived from the deep layer of the submucous tissue of the mouth and pharynx, and can be seen passing down channels in the bone just beneath the convoluted layer of these mucous glands (Plate 6, fig. 19).

These invasions of the enamel organ by prolongations of glandular tissue from the deep surface of the epithelium of the mouth were best seen in sections taken from the intermaxillary bones.

This enamel organ, entirely made up of blood-vessels enclosed in sheaths and surrounded by well defined tubular glands, I have found only in *Tautoga*—in the other Labridæ examined, although there is a distinct secretory apparatus, there is no such definite glandular structure, and in neither of them could I find any sign of penetration of this tissue from without.

I have provisionally made use of the term "gland" in describing the enamel organ of *Tautoga*, because, however anomalous it may appear, these bodies in the enamel organ have all the histological appearances of simple tubular glands, and their direct communication with the unmistakable glandular tissue of the mouth is most clearly shown in numerous preparations.




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*Halichœres Poœcilopterus.*—In this species the blood-vessels in the enamel organ, instead of being confined to narrow tubes, expand into large dilatations or sinuses, and fill up the concavities or hollows in a system of somewhat irregularly arranged, branched tube-like formations, with double walls, which form a kind of "key pattern" around the enamel organ (Plate 6, fig. 22).

Between the blood sinuses and the tubular processes there is no intervening tissue enclosing nucleated cells as in *Sargus*, but they are apparently in close contact. This arrangement would suggest that the secreting organ is either in the space enclosed between the tube walls or within these walls themselves, especially as at the free margin of the enamel organ numerous fine radiating processes extend from them into the stroma—these would appear to be the ducts or channels by which the secreted substance is conveyed to the stroma.

The actual structure of these thick-walled tubes is very difficult to interpret. In the reticular tissue between the tube walls, which is generally arranged diagonally, I was unable to detect any cells; the tube walls, which look thick and somewhat indistinct in the thinnest sections, appear to be made up of ovoid bodies arranged longitudinally, and suggest a similar structure to that of the tubes of *Pseudolabrus japonicus*.

Minute processes extend from these tubes to the surface which is continuous with the stroma, which in these preparations is finely granular, and shows no indication of fibrillation. Just within the stroma a layer of scattered bodies is seen, which take the stain faintly; they are of various shapes and sizes as in *Sargus* and show no nuclei, and are apparently little masses of the calcifying substance elaborated by the enamel organ, corresponding to the similar bodies seen in *Sargus*, and also in mammalian enamel organs.

The calcifying enamel, which in this preparation fortunately remained attached to the stroma, shows a distinct tubular structure, and also rounded globules within its substance, and, as in the forming enamel of *Sargus* (fig. 12), there is no appearance of any large tubes entering it, and the delicate fibrillation seen in *Sargus* is not here visible—but it is possibly obscured by the dense granular tissue.

The blood-vessels are much larger and more abundant than in the other fish examined; at the sides of the enamel organ especially, they occupy very wide spaces between the tubes. In several preparations, the row of processes from the lower part of the curved tubes (fig. 22, *dt*) forms a thick continuous band, having very much the appearance of a row of cells, and it was only by examining many specimens that I was able to be certain they were not cellular; when, however, they are cut in the right plane, they appear as in fig. 22, and a key to their nature is obtained in sections of *Pseudolabrus*, where they form a much more definite and regular layer (fig. 20, *dt*). In some slides of *Halichœres*, where a very little enamel is laid down, it is seen as twisted, wavy fibres, which seem to form the basis of the first formed enamel, and it would appear that these delicate fibres are present in the stroma, although obscured by the dense granular deposit.

*Pseudolabrus japonicus.*—The enamel organ of this fish, while showing some resemblance to that of *Halichœres*, also shows some distinct differences of structure.

The blood-vessels are not so largely developed as in *Halichœres*, and are enclosed in a narrow sheath like those in *Tautoga*, but the tubes of the enamel organ contain distinct rows of large cell-like bodies (fig. 20); these tubes surround the blood-vessels, and are very easily separated from them (fig. 21), leaving the vascular tubes projecting. The fine fibres or channels which appear to correspond to the ducts in *Tautoga* form a very conspicuous part of the enamel organ, and extend in a radiating manner from the tubes of the enamel organ to the stroma, which is also here seen *in situ* (fig. 20). The stroma is finely granular, as in *Halichœres*, and similar irregular stained bodies are seen within it.

It is somewhat remarkable that in the same group of fish there should be such marked differences in the structure of the enamel organ. While, however, *Tautoga* shows the most distinct glandular form, the same end would seem to be obtained by the arrangement of the tissue in the two other species.

The process of enamel formation would appear to be one of true secretion, the glands in *Tautoga* and the tubes in the Japanese fish serving the same function, that of separating the lime salts from the circulating blood to form the calcified enamel, the organic matrix of which is formed by the transformed ameloblasts which have become converted into the stroma, as shown by Mr. Tomes in the Gadidæ. This function of the glands and tubes would, however, explain the anomaly referred to by this author in his paper (1A) (at least so far as the two families described are concerned), that the lime salts are deposited at so great a distance from the blood-vessels. Instead of being elaborated at a distance from the point of deposition, the blood-vessels and the cells which are in connection with them being in direct contact with the stroma in which the enamel is undergoing calcification, the separation of the salts from the blood and their conveyance to the seat of calcification would take place within the enamel organ itself.

No definite conversion of the whole enamel organ into a system of glands and blood-vessels has, I think, been hitherto described. Leon Williams (4), however, in his paper "On the Formation and Structure of Dental Enamel," describes a secreting structure in the cells of the stratum intermedium of the Rat—in the stages when the stellate reticulum has disappeared, and the blood-vessels are in contact with the cells of the stratum intermedium, and he considers that at all events the cells of this layer should be classed among the true secreting tissues. His description and photographs certainly appear to indicate that at all events in the Rat there is a survival of the secretory apparatus so highly developed in the fishes. In the Rat, the photographs show that the cells of the stratum intermedium separate from the blood-vessels when pulled apart, in the same manner as do the enamel organ tubes from the vessels in *Pseudolabrus*. Prof. Poulton described blood-vessels in the stellate reticulum of the Rat in 1888 (5), but while the presence of

blood-vessels in the enamel organ of these fish is a regular and constant phenomenon, it has so far been only detected in a rudimentary condition in Mammalia.

Blood-vessels have been described also in the stellate reticulum of the Wallaby by Maretт Tims and Hopewell-Smith (6), and they may be seen in the same situation in many specimens of *Macropus*.

This condition in fish would seem to elucidate to some extent the whole problem of enamel formation. It would appear that both the conversion and the secretion theories are reconcilable, the conversion of the ameloblasts into an organic stroma favouring the former view, while the existence of a definite secreting apparatus in the enamel organ confirms the latter. In Marsupials, it is only the portion of the ameloblast cell prolonged into the Tomes' process that enters into the organic fibrillar basis of the enamel, while the ameloblast cell itself, and most probably the cells of the stratum intermedium, serve to separate the lime salts from the blood, as do the gland-like bodies and tubes in the fish, and in the higher Mammalia the Tomes' processes, although much less pronounced, are incorporated in the forming enamel. It is curious that, in the early stages of development in these fish, the ameloblasts seem to exercise their functions in the same manner as in Mammalia. From the evolutionary standpoint this seems a very puzzling problem.

In the placoid scales of Elasmobranchs, which are considered to show the first formation of enamel in the Vertebrates, this tissue is deposited by definite ameloblasts (7), but, although in the fishes described a thin layer of enamel is first laid down by the ameloblasts, the bulk of it is calcified by a definite secreting organ which has apparently taken up the functions of the ameloblasts.

In many fish and in the higher Vertebrata the ameloblasts persist throughout the period of enamel formation, and the whole thickness of the tissue is calcified by their agency.

My attention has been drawn, since writing this paper, to a very similar arrangement of the secreting organ to that found in *Sargus*, in the lime-secreting glands of the Earthworm.

These were first described by Julius Leo in 1820, and have been more recently studied by Darwin (8), Beddard (9), and others, their histological structure having been carefully investigated by Harrington (10), who especially demonstrated the relations of the blood-vessels to these glands.

He showed that the second and third pairs of glands in the 11th and 12th somites of *Lumbricus* showed a glandular tissue arranged on either side of radially placed blood sinuses, and that the lime was there secreted, passing forward through the anterior gland to be discharged into the œsophagus through the anterior foramen.

The arrangement of the gland tissue along the walls of the blood sinuses, as shown in the drawings in Mr. Harrington's paper, has a remarkable similarity to the arrangement seen in the enamel organ of *Sargus*, the vascular tubes or sinuses in the latter apparently corresponding to the blood sinuses in *Lumbricus*.

In the worm, however, the forward extension of the glandular organ to the point of discharge into the œsophagus itself forms the gland duct, the secreting process not being continued in the anterior glands, while in the fish there is an intervening tubular structure between each pair of blood sinuses which apparently serves as the gland duct. (Plate 5, figs. 10 and 11.)

## Summary.

The principal points of interest in this investigation are—

That the markings from without in the enamel of the fish described are true tubes, and stain from without when treated with alcoholic fuschin stains, which are allowed to enter by capillary attraction.

That in *Sargus* the unerupted teeth show very wide stained areas, in which the tubes lie, and that these areas eventually become calcified.

That this condition suggests that the tubes have a calcifying function, conveying the lime salts to the forming enamel, which is still further indicated by the presence of granules in the enamel tubes.

That the pattern formed by the arrangement of the enamel prisms is quite distinct from that produced by the tubes, and shows a very complex structure in the area near the dentine.

That in some species of *Sargus* there is a very well marked system of stained tubes from the dentine, which pass all across the enamel in *Sargus noct*, and communicate with those from without, and the enamel is penetrated in different degrees by the dentinal tubes in different species.

That a similar staining from without is obtained in the enamel of *Chrysophrys*, *Pagellus*, and the Labridæ, the most complete and complicated system of tubes from without being seen in *Scarus*.

That the part of the enamel in *Sargus* near the dentine is laid down by true ameloblast cells, the rest of the enamel being formed after these cells have disappeared and the tubes and stroma have taken their place, and that the part of the enamel bordering the dentine contains no tubes from without.

That in both *Sargus* and *Labrus*, blood-vessels enter the enamel organ, which run in definite sheaths, and alternate regularly with the secreting organ.

That in the Labridæ there is further evidence of the conversion of the enamel organ into a true secreting structure, which in *Tautoga* assumes the form of tubular glands with distinct central ducts—these glands surrounding the penetrating blood-vessels.

That in the different species of the Labridæ there is a considerable difference in the structure and arrangement of this secreting organ.

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## DESCRIPTION OF PLATES.

Lettering applicable to all the figures.

| | | | |
|---|---|---|---|
| *am.* | Ameloblasts. | *e.o.* | Enamel organ. |
| *b.* | Blood-vessels. | *ext.* | External epithelium. |
| *c.* | Capsule. | *g.* | Glandular tissue. |
| *d.* | Dentine. | *od.* | Odontoblasts. |
| *dt.* | Ducts. | *s.* | Stroma. |
| *e.* | Enamel. | *sp.* | Calcospherites. |
| *e′.* | Enamel area not penetrated by external tubes. | *t.* | Tubes of enamel organ. |

### Plate 5.

Fig. 1.—Ground section of unerupted molar tooth of *Sargus noct*, stained in bulk with alcoholic fuchsin by the capillary attraction method, before grinding. The dried remains of the enamel organ are seen at *e.o.*, very deeply stained, the stain passes into the enamel in broad vertical bands, which communicate with the broader horizontal coloured band, but the stain does not penetrate into the layer of enamel at *e′*. The tubes from the dentine are strongly stained. From a photograph. × 50.

Fig. 2.—Ground section of a slightly further advanced tooth of *Sargus noct*, treated as in fig. 1. The horizontal band has disappeared, but the broad vertical coloured bands are still seen. From a photograph. × 50.

Fig. 3.—From a similarly treated section of the completed enamel of *Sargus noct*. The majority of the stained tubes are cut off from communication with the exterior of the enamel in *Sargus noct*, where a tubular system from the dentine is fully developed. From a photograph. × 50.

Fig. 4.—Ground section of a fuschin-stained, fully erupted tooth of *Sargus ovis*, preserved in formalin. The stained tubes are seen communicating with the exterior—the stain penetrates the finest divisions, but does not enter the area of enamel at *e′*. × 50.

Fig. 4A.—From a similar preparation, showing granules in the tubes. From a photograph. × 200.

Fig. 5.—Stained enamel and dentine of *Sargus vulgaris*, dry preparation, showing penetration of dentinal tubes, but much less deeply than in *Sargus noct*. From a photograph. × 50.

Fig. 6.—Stained ground section of upper pharyngeal tooth of *Scarus*. The stain has penetrated the vertically arranged tubes, but did not reach the horizontal ones. From a photograph. × 50.

Fig. 7.—Stained ground section of intermaxillary tooth of *Labrus* (*Tautoga onitis*). The tubes branch much nearer the enamel surface than in *Sargus*, and the terminal branches are much finer. From a photograph. ×50.

Fig. 8.—Tooth germ of *Sargus ovis*, preserved whilst fresh, in formalin, paraffin section somewhat displaced, no tubes are seen in the enamel organ, and the ameloblasts are in contact with the formed, but uncalcified, non-tubular enamel. The dentine pulp is very large, and no dentine is formed, an indication of a commencing reticulate stroma seen at *s*. Drawing with Abbé drawing prism. ×30.

Fig. 9.—A later germ of *Sargus ovis* (from the same material). The ameloblasts and the cells of the external epithelium have entirely disappeared, and a tubular tissue and stroma have taken their place. A small amount of calcified enamel remains in connection with the stroma—the part of the enamel formed by the ameloblasts next the dentine has now been removed by the decalcifying acid, and a large area of dentine is formed. The darker tubes contain blood-vessels, and the delicate tubes of the enamel organ, not well shown in the paraffin section, lie between them. Drawing (Abbé prism). ×30.

Fig. 10.—Enamel organ of *Sargus ovis*. Showing vascular tubes in connection with the blood-vessels of the capsule, enamel organ tubes and stroma. A photograph. ×130.

Fig. 11.—A portion of the enamel organ of *Sargus ovis*, more highly magnified, showing the connection of the tubular organ with the vascular tubes and the intermediate nucleated cells. Drawing (Abbé prism). ×290.

Fig. 12.—A portion of calcifying enamel in connection with the stroma in *Sargus ovis*. Drawing (Abbé prism). ×200.

## Plate 6.

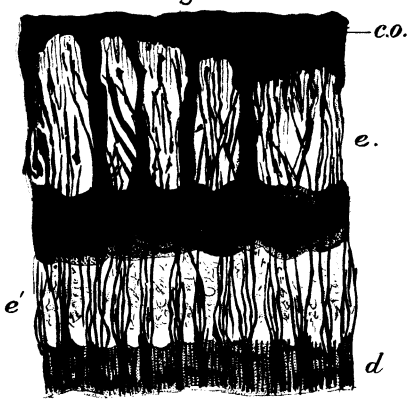
Fig. 13.—Early germ of *Labrus* (*Tautoga onitis*). A small amount of enamel is laid down under the ameloblasts. Glandular tissue is seen at the summit of the enamel organ spreading into the external epithelium. From a photograph. ×35.

Fig. 14.—A germ of *Tautoga onitis*, showing the vascular enamel organ and remains of the stroma between it and the enamel. From a photograph. ×30.

Fig. 15.—An early germ of *Tautoga*, showing a mass of glandular tissue entering the capsule and spreading around the ameloblast layer in the position of the external epithelium. From a photograph. ×35.

Fig. 16.—The fully formed glandular enamel organ of *Tautoga onitis*—tubular glands are seen with central ducts opening on the inner surface of the enamel organ, where they communicate with the stroma, which, being

Fig 1.

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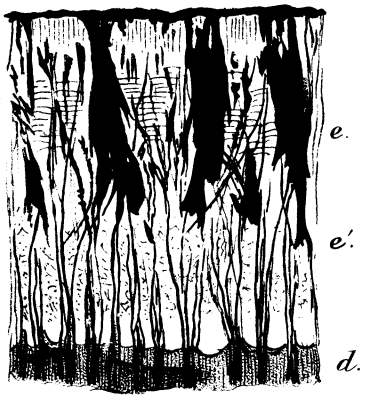


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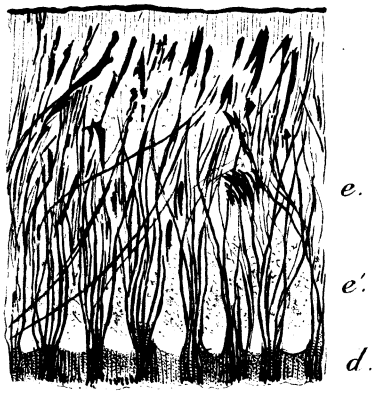


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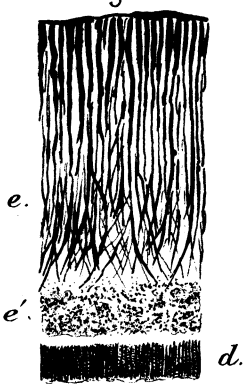


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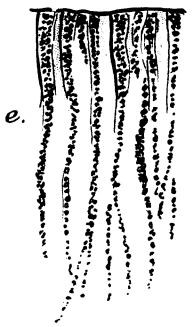


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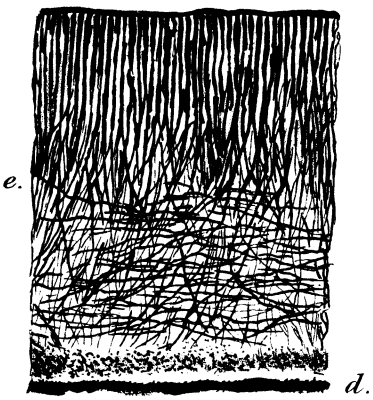


Fig. 7.


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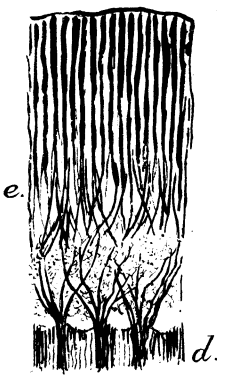


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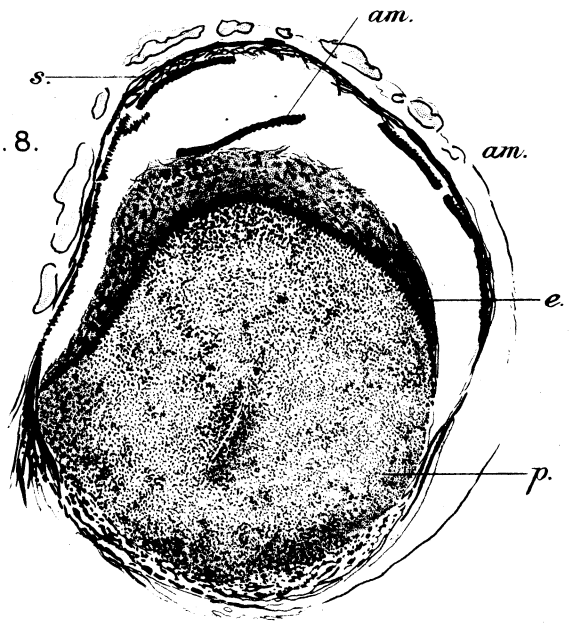


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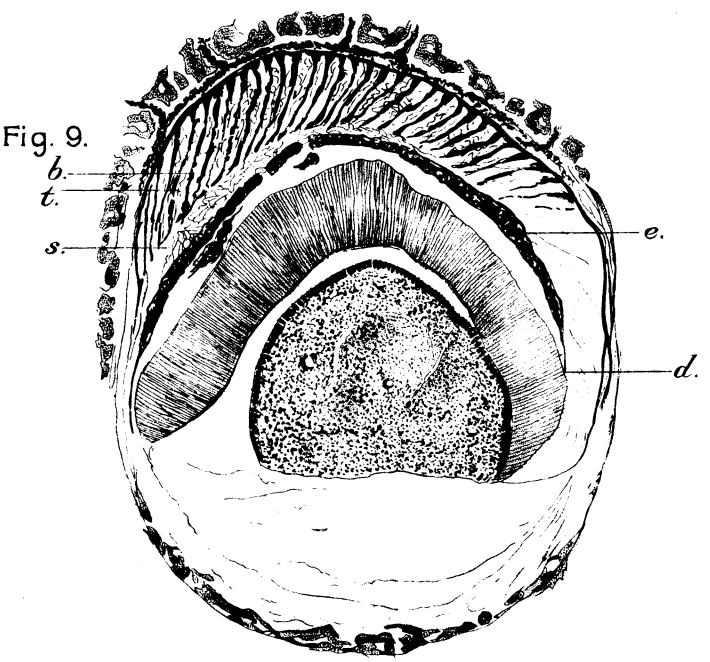


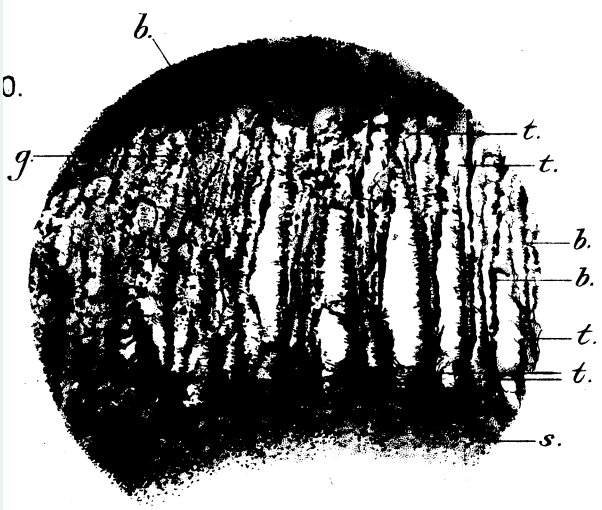


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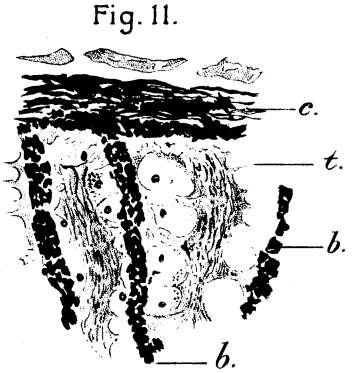


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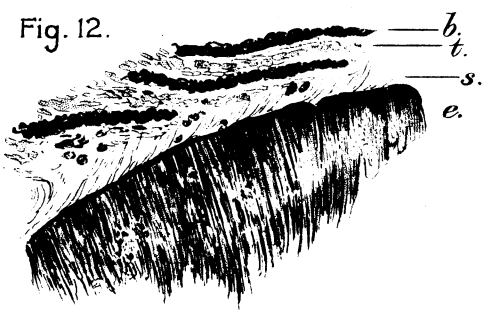

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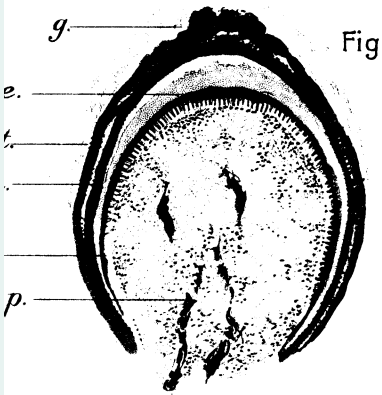


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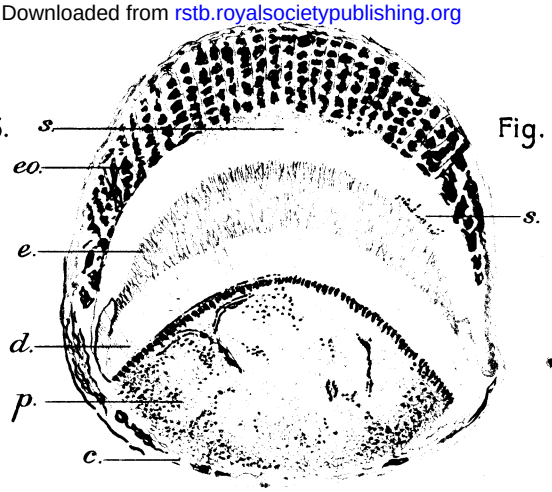


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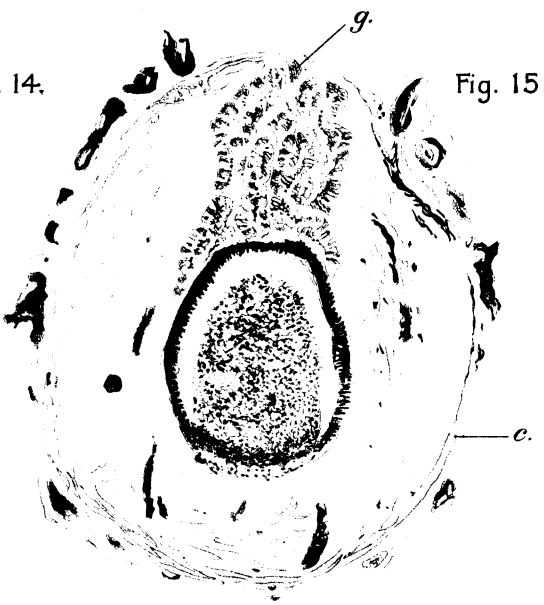
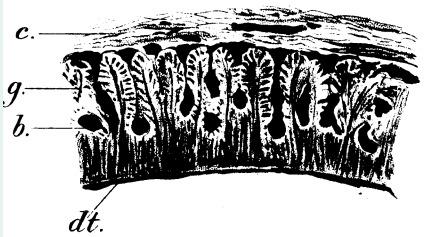
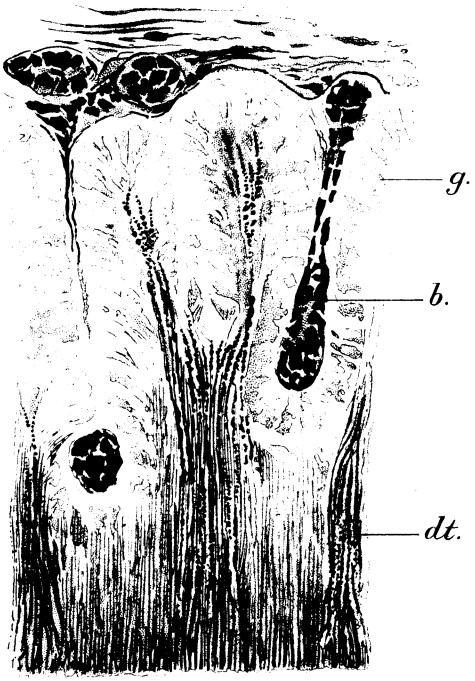



Fig. 15.

Fig. 16.

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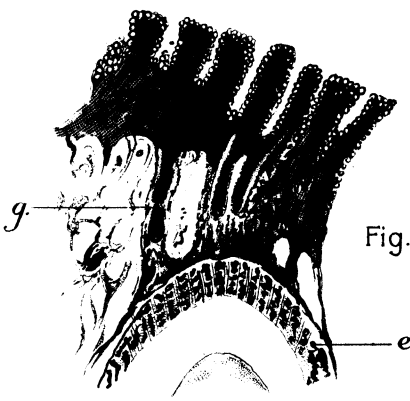


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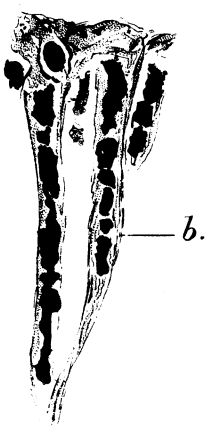


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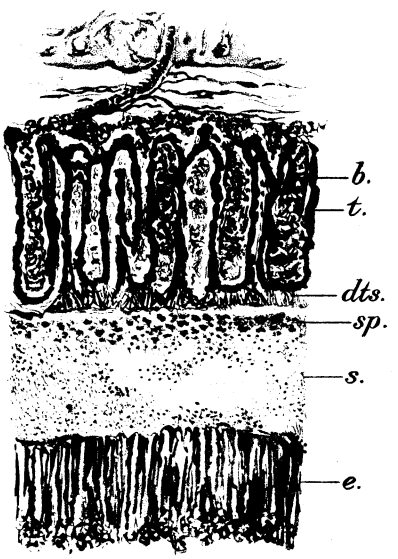


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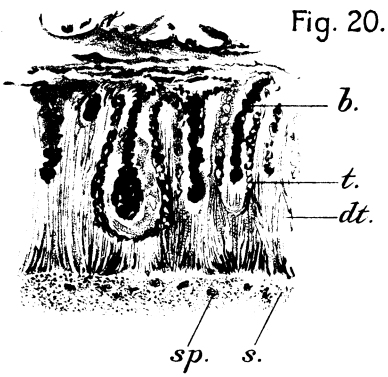


Fig. 20.

Fig. 21.

very delicate, has become detached in the preparation—the dark areas are the vascular tubes. From a photograph. ×110.

Fig. 17.—A portion of fig. 16, highly magnified, showing the ducts of the glands with their granular contents. Drawing (Abbé prism). ×700.

Fig. 18.—Two detached vascular tubes from the enamel organ of *Tautoga onitis*. From a photograph. ×500.

Fig. 19.—From the pharyngeal surface of *Tautoga onitis*, showing the mucous glands above and the glandular prolongations from the deeper part of the epithelium passing along channels in the bone to the enamel organ at *e.o.* Drawing (Abbé prism). ×30.

Fig. 20.—The enamel organ of a Japanese *Labrus*, *Pseudolabrus japonicus*, showing convoluted tubes surrounding the blood-vessels and delicate processes radiating to the stroma at *s.* and at *sp.* calcospherites, which are faintly stained. The stroma, which is granular, remains in connection with the rest of the enamel organ in this specimen. Drawing (Abbé prism). ×125.

Fig. 21.—Showing the tubes of the enamel organ pulled away from the intervening blood-vessels—viewed somewhat obliquely. Drawing (Abbé prism). ×125.

Fig. 22.—Enamel organ, stroma and calcifying enamel of *Halichœres poœcilopterus* (a Japanese *Labrus*). The blood-vessels are very large and abundant, and numerous delicate processes pass from the convoluted tubes of the enamel organ to the stroma. It is noticeable that the enamel is markedly tubular, although no tubes are seen to pass into it. Drawing (Abbé prism). ×125.



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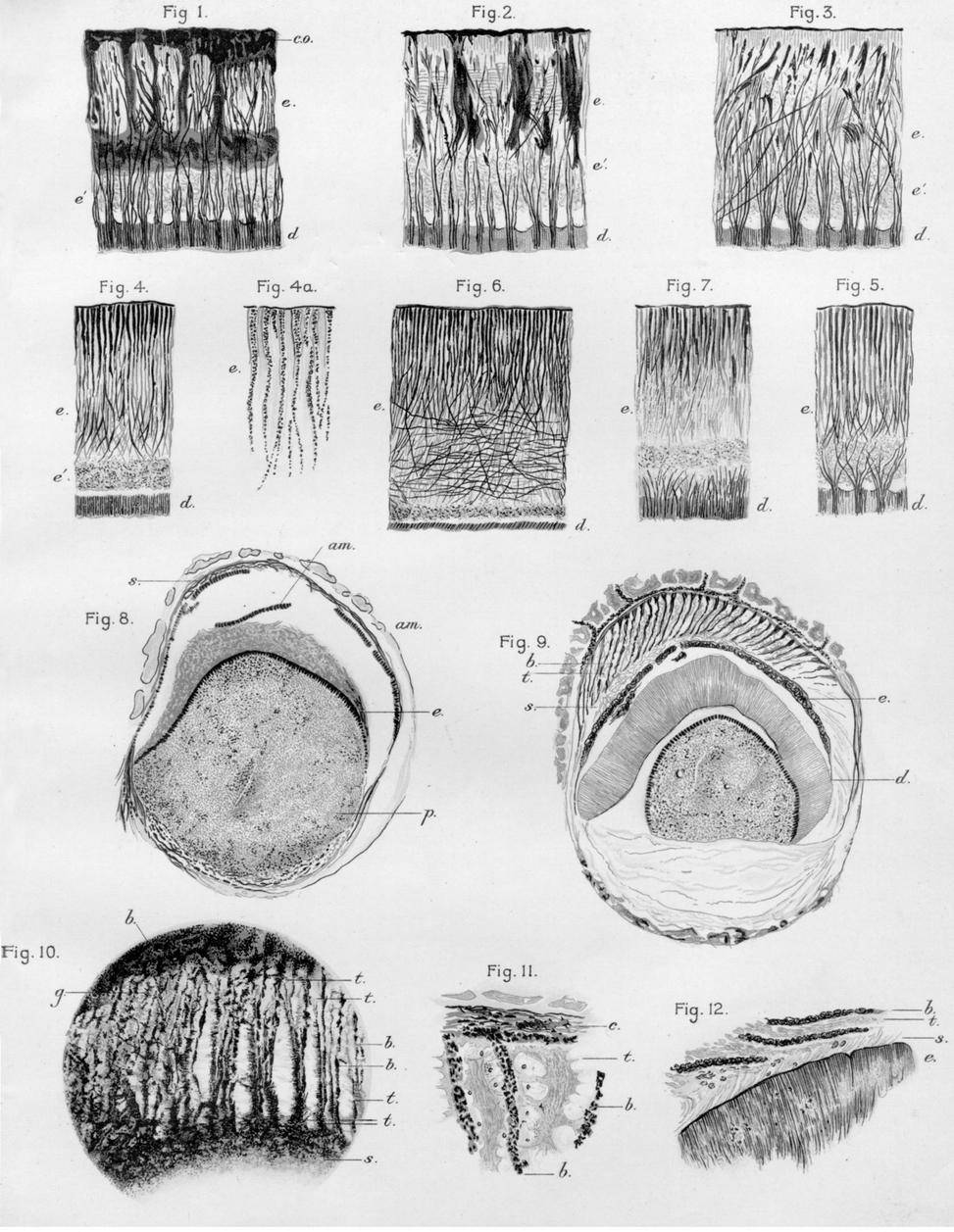


PLATE 5.

Fig. 1.—Ground section of unerupted molar tooth of *Sargus noct*, stained in bulk with alcoholic fuchsin by the capillary attraction method, before grinding. The dried remains of the enamel organ are seen at *e.o.*, very deeply stained, the stain passes into the enamel in broad vertical bands, which communicate with the broader horizontal coloured band, but the stain does not penetrate into the layer of enamel at *e'*. The tubes from the dentine are strongly stained. From a photograph. ×50.

Fig. 2.—Ground section of a slightly further advanced tooth of *Sargus noct*, treated as in fig. 1. The horizontal band has disappeared, but the broad vertical coloured bands are still seen. From a photograph. ×50.

Fig. 3.—From a similarly treated section of the completed enamel of *Sargus noct*. The majority of the stained tubes are cut off from communication with the exterior of the enamel in *Sargus noct*, where a tubular system from the dentine is fully developed. From a photograph. ×50.

Fig. 4.—Ground section of a fuschin-stained, fully erupted tooth of *Sargus ovis*, preserved in formalin. The stained tubes are seen communicating with the exterior—the stain penetrates the finest divisions, but does not enter the area of enamel at *e'*. ×50.

Fig. 4A.—From a similar preparation, showing granules in the tubes. From a photograph. ×200.

Fig. 5.—Stained enamel and dentine of *Sargus vulgaris*, dry preparation, showing penetration of dentinal tubes, but much less deeply than in *Sargus noct*. From a photograph. ×50.

Fig. 6.—Stained ground section of upper pharyngeal tooth of *Scarus*. The stain has penetrated the vertically arranged tubes, but did not reach the horizontal ones. From a photograph. ×50.

Fig. 7.—Stained ground section of intermaxillary tooth of *Labrus* (*Tautoga onitis*). The tubes branch much nearer the enamel surface than in *Sargus*, and the terminal branches are much finer. From a photograph. ×50.

Fig. 8.—Tooth germ of *Sargus ovis*, preserved whilst fresh, in formalin, paraffin section somewhat displaced, no tubes are seen in the enamel organ, and the ameloblasts are in contact with the formed, but uncalcified, non-tubular enamel. The dentine pulp is very large, and no dentine is formed, an indication of a commencing reticulate stroma seen at *s*. Drawing with Abbé drawing prism. ×30.



Fig. 9.—A later germ of *Sargus ovis* (from the same material). The ameloblasts and the cells of the external epithelium have entirely disappeared, and a tubular tissue and stroma have taken their place. A small amount of calcified enamel remains in connection with the stroma—the part of the enamel formed by the ameloblasts next the dentine has now been removed by the decalcifying acid, and a large area of dentine is formed. The darker tubes contain blood-vessels, and the delicate tubes of the enamel organ, not well shown in the paraffin section, lie between them. Drawing (Abbé prism). ×30.

Fig. 10.—Enamel organ of *Sargus ovis*. Showing vascular tubes in connection with the blood-vessels of the capsule, enamel organ tubes and stroma. A photograph. ×130.

Fig. 11.—A portion of the enamel organ of *Sargus ovis*, more highly magnified, showing the connection of the tubular organ with the vascular tubes and the intermediate nucleated cells. Drawing (Abbé prism). ×290.

Fig. 12.—A portion of calcifying enamel in connection with the stroma in *Sargus ovis*. Drawing (Abbé prism). ×200.

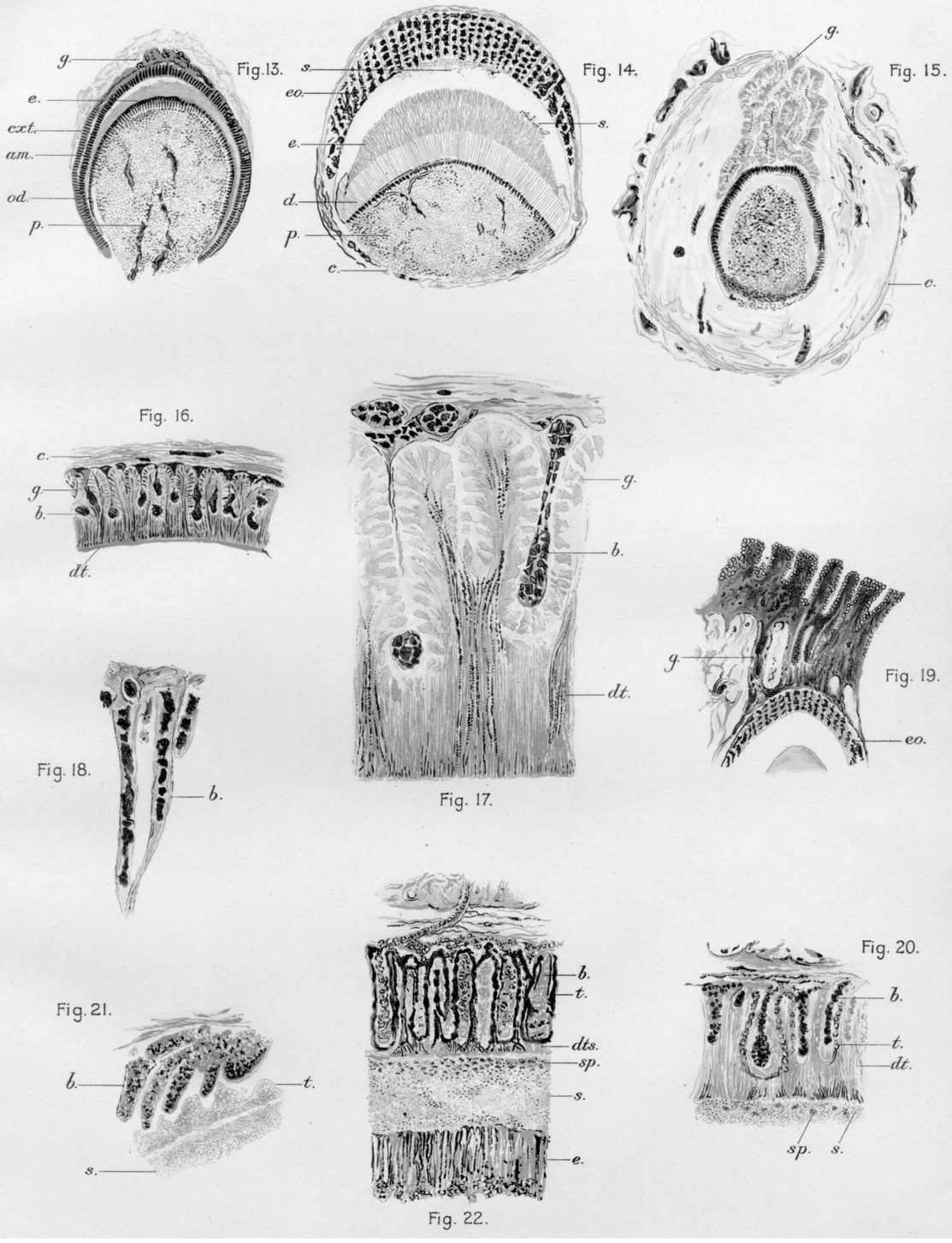


PLATE 6.

Fig. 13.—Early germ of *Labrus* (*Tautoga onitis*). A small amount of enamel is laid down under the ameloblasts. Glandular tissue is seen at the summit of the enamel organ spreading into the external epithelium. From a photograph. ×35.

Fig. 14.—A germ of *Tautoga onitis*, showing the vascular enamel organ and remains of the stroma between it and the enamel. From a photograph. ×30.

Fig. 15.—An early germ of *Tautoga*, showing a mass of glandular tissue entering the capsule and spreading around the ameloblast layer in the position of the external epithelium. From a photograph. ×35.

Fig. 16.—The fully formed glandular enamel organ of *Tautoga onitis*—tubular glands are seen with central ducts opening on the inner surface of the enamel organ, where they communicate with the stroma, which, being very delicate, has become detached in the preparation—the dark areas are the vascular tubes. From a photograph. ×110.

Fig. 17.—A portion of fig. 16, highly magnified, showing the ducts of the glands with their granular contents. Drawing (Abbé prism). ×700.

Fig. 18.—Two detached vascular tubes from the enamel organ of *Tautoga onitis*. From a photograph. ×500.



Fig. 19.—From the pharyngeal surface of *Tautoga onitis*, showing the mucous glands above and the glandular prolongations from the deeper part of the epithelium passing along channels in the bone to the enamel organ at *e.o.* Drawing (Abbé prism). ×30.

Fig. 20.—The enamel organ of a Japanese *Labrus*, *Pseudolabrus japonicus*, showing convoluted tubes surrounding the blood-vessels and delicate processes radiating to the stroma at *s.* and at *sp.* calcospherites, which are faintly stained. The stroma, which is granular, remains in connection with the rest of the enamel organ in this specimen. Drawing (Abbé prism). ×125.

Fig. 21.—Showing the tubes of the enamel organ pulled away from the intervening blood-vessels—viewed somewhat obliquely. Drawing (Abbé prism). ×125.

Fig. 22.—Enamel organ, stroma and calcifying enamel of *Halichæres pœcilopterus* (a Japanese *Labrus*). The blood-vessels are very large and abundant, and numerous delicate processes pass from the convoluted tubes of the enamel organ to the stroma. It is noticeable that the enamel is markedly tubular, although no tubes are seen to pass into it. Drawing (Abbé prism). ×125.